(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/06990 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US00/40370

(22) International Filing Date: 13 July 2000 (13.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/360,020 23 July 1999 (23.07.1999) US
09/589,476 7 June 2000 (07.06.2000) US

(71) Applicant (*for all designated States except US*): THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 12th Floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): CARSON, Dennis, A. [US/US]; 14824 Vista del Oceano, Del Mar, CA 92014 (US). COTTAM, Howard, B. [US/US]; 1890 Lendee Drive, Escondido, CA 92025 (US). ADACHI, Souchi [JP/US]; 8223 Caminito Modena, La Jolla, CA 92037 (US). LEONI, Lorenzo, M. [CH/US]; 3977 Camino Calma, San Diego, CA 92122 (US).

(74) Agent: VIKSNINS, Ann, S.; Schwegman, Lundberg, Woessner & Kluth, P.O. Box 2938, Minneapolis, MN 55402 (US).

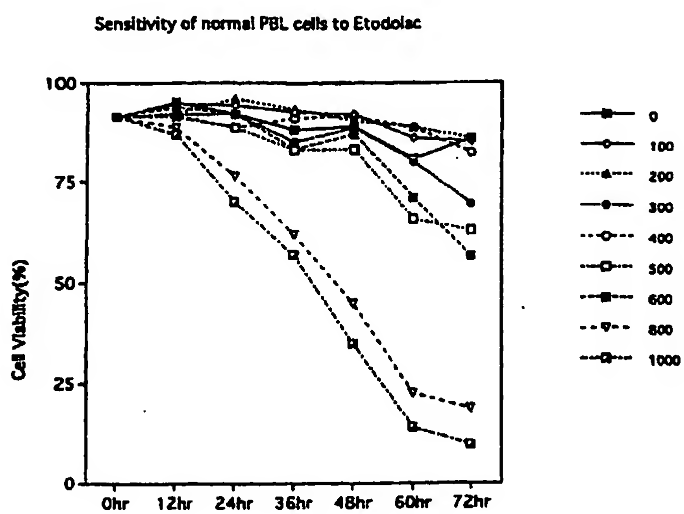
(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF ETODOLAC TO TREAT CANCER



(57) Abstract: A method of treating multiple myeloma (MM) is provided comprising administering an amount of etodolac to a subject afflicted with MM that is effective to selectively reduce the viability of and/or sensitize the cancer cells to an anti-cancer agent.

WO 01/06990 A2

USE OF ETODOLAC TO TREAT CANCER

Cross-Reference to Related Application

This application is a continuation-in-part of U.S. patent application Serial No. 09/360,020 filed July 23, 1999, pending.

Background of the Invention

The invention was made with Government support under Grant No. 5ROI GM23200-24 awarded by the National Institute of Health. The Government has certain rights in the invention.

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the United States. CLL involves the cancerous proliferation of lymphocytes. It is most common among older adults; 90 percent of the cases are in people more than 50 years old. It occurs 1-3 times more often among men than among women. The onset of CLL tends to be insidious, with symptoms developing gradually. Because it involves an overproduction of mature, functional lymphocytes, persons with this disorder may survive for years. In contrast, in some, the disorder proceeds very rapidly, and requires immediate treatment. Currently, the adenine deoxynucleosides fludarabine (fludara) and 2-chlorodeoxyadenosine (2CdA) are the drugs of choice to treat the disease. However, clinical remissions are seldom induced, and patients eventually succumb from their leukemia.

Multiple myeloma is a cancer in which malignant plasma cells accumulate in the bone marrow and produce an immunoglobulin, usually monoclonal IgG or IgA. Common complications of overt multiple myeloma include recurrent bacterial infections, anemia, osteolytic lesions, and renal insufficiency. Multiple myeloma is responsible for about 1 percent of all cancer-related deaths in Western countries. Its epidemiologic pattern remains obscure, and its cause is unknown. D. A. Reidel et al., Hematol. Oncol. Clin. North Am., 6, 225 (1992).

Melphalan, cyclophosphamide, and glucocorticoids are the most effective drugs against multiple myeloma; the classic combination of melphalan and prednisone is still the standard treatment for most patients. R. Alexanian et al.,

N. Engl. J. Med., 330, 484 (1994). Combinations of other drugs, including vinca alkaloids, nitrosoureas, and anthracyclines, are active against myeloma but are no more effective than melphalan and prednisone. R. Alexanian et al., N. Engl. J. Med., 330, 484 (1994). Results with novel agents like purine analogues or taxane derivatives have not been promising. The regimen of vincristine, doxorubucin, and dexamethasone (VAD) – or a similar combination, with high-dose methylprednisolone substituted for high-dose dexamethasone (VAMP) – which is often used in patients with newly diagnosed disease, has not prolonged survival more than other regimens in a randomized clinical trial; its main advantage is the rapid induction of remission. R. Alexanian et al., N. Engl. J. Med., 330, 484 (1994).

The lack of progress with conventional chemotherapy has heightened interest in high-dose therapy. Treatment with melphalan in high doses (140 mg per square meter of body-surface area, given intravenously without hematopoietic support) can induce complete remissions in 20 to 30 percent of patients (including disappearance of the M component), but it causes severe and sometimes irreversible myelosuppression. The rate of death due to the toxicity of this treatment is approximately 10 percent in patients with newly diagnosed disease and more than 20 percent in previously treated patients. D. Cunningham et al., J. Clin. Oncol., 12, 764 (1994); J. L. Harcovseau et al., Blood, 79, 2827 (1992); B. Barlogle et al., Blood, 72, 2015 (1988).

Transplantation of autologous hematopoietic stem cells (obtained from bone marrow or blood) accelerates the restoration of hematopoiesis after the administration of high dose melphalan and allows for a combination of intensive chemotherapy and total-body irradiation or for the use of higher doses of melphalan (200 mg per square meter). Autologous stem-cell transplantation may be a useful form of salvage therapy for patients whose disease is refractory to initial standard chemotherapy and for patients in relapse with chemosensitive myeloma, but it has limited value for patients in relapse with resistant myeloma. Other therapies for multiple myeloma are discussed in R. Bataille et al., N. Engl. J. Med., 336, 1657 (1997).

The number of nonsteroidal anti-inflammatory drugs (NSAIDs) has increased to the point where they warrant separate classification. In addition to

aspirin, the NSAIDs available in the U.S. include meclofenamate sodium, oxyphenbutazone, phenylbutazone, indomethacin, piroxicam, sulindac and tolmetin for the treatment of arthritis; mefenamic acid and zomepirac for analgesia; and ibuprofen, fenoprofen and naproxen for both analgesia and arthritis. Ibuprofen, mefenamic acid and naproxen are used also for the management of dysmenorrhea.

The clinical usefulness of NSAIDs is restricted by a number of adverse effects. Phenylbutazone has been implicated in hepatic necrosis and granulomatous hepatitis; and sulindac, indomethacin, ibuprofen and naproxen with hepatitis and cholestatic hepatitis. Transient increases in serum aminotransferases, especially alanine aminotransferase, have been reported. All of these drugs, including aspirin, inhibit cyclooxygenase, that in turn inhibits synthesis of prostaglandins, which help regulate glomerular filtration and renal sodium and water excretion. Thus, the NSAIDs can cause fluid retention and decrease sodium excretion, followed by hyperkalemia, oliguria and anuria. Moreover, all of these drugs can cause peptic ulceration. See, Remington's Pharmaceutical Sciences, Mack Pub. Co., Easton, PA (18th ed., 1990) at pages 1115-1122.

There is a large amount of literature on the effect of NSAIDs on cancer, particularly colon cancer. For example, see H. A. Weiss et al., Scand J. Gastroent., 31, 137 (1996) (suppl. 220) and Shiff et al., Exp. Cell Res., 222, 179 (1996). More recently, B. Bellosillo et al., Blood, 92, 1406 (1998) reported that aspirin and salicylate reduced the viability of B-cell CLL cells *in vitro*, but that indomethacin, ketoralac and NS-398, did not.

C. P. Duffy et al., Eur. J. Cancer, 34, 1250 (1998), reported that the cytotoxicity of certain chemotherapeutic drugs was enhanced when they were combined with certain non-steroidal anti-inflammatory agents. The effects observed against human lung cancer cells and human leukemia cells were highly specific and not predictable; i.e., some combinations of NSAID and agent were effective and some were not. The only conclusion drawn was that the effect was not due to the cyclooxygenase inhibitory activity of the NSAID.

The Duffy group filed a PCT application (WO98/18490) on October 24, 1997, directed to a combination of a "substrate for MRP", which can be an anti-

cancer drug, and a NSAID that increases the potency of the anti-cancer drug. NSAIDs recited by the claims are acemetacin, indomethacin, sulindac, sulindac sulfide, sulindac sulfone, tolmetin and zomepirac. Naproxen and piroxicam were reported to be inactive.

Nardella et al. (PCT/WO/00/02555) disclose that the level of leukemic lymphocytes in patients suffering from chronic lymphocytic leukemia (CLL) is reduced by the administration of the NSAID etodolac. See also, F. A. Nardella et al., Arthritis and Rheumatism, 42, S56 Abstract 41 (Sept. 1999).

Therefore, a continuing need exists for methods to control cancers, such as leukemias, and to increase the potency of anti-cancer drugs with relatively non-toxic agents.

Summary of the Invention

In one aspect, the present invention provides a therapeutic method to treat multiple myeloma (MM), comprising administering to a mammal afflicted with MM an amount of etodolac, R(−) etodolac, or an analog thereof, effective to inhibit the viability of the cancerous cells of said mammal. The viability of the cancerous bone marrow cells is reduced selectively, while maintaining the viability of normal cells.

The present invention also provides a method of increasing the susceptibility of human cancer cells, e.g., solid tumor or leukemia cells, such as chronic lymphocytic leukemia (CLL) cells or MM cells, to a chemotherapeutic agent comprising contacting the cells with an effective sensitizing amount of etodolac, or an analog thereof. Thus, the invention provides an improved therapeutic method for the treatment of a human or other mammal afflicted with a solid tumor such as a lymphoma or prostate cancer or with CLL or MM, wherein an effective amount of etodolac or an analog thereof is administered to an afflicted subject undergoing treatment with one or more chemotherapeutic ("antineoplastic") agents, wherein the cancerous cells are rendered more susceptible to the chemotherapeutic agent(s).

Preferably, the R(-) isomer of etodolac is administered in conjunction with one or more chemotherapeutic agents effective against CLL or MM such as fludarabine (fludara) or 2-chlorodeoxyadenosine (2CdA) (for CLL) or melphalan, cyclophosphamide or interferon-α (for MM). Melphalan can be

given in doses of about 140-200 mg/m^2, optionally in combination with total body irradiation, followed by bone marrow or stem cell transplantation, as discussed in R. Bataille et al., N. Eng. J. Med., 336, 1657 (1997). Unexpectedly, the R(-) isomer of etodolac, which exhibits little anti-inflammatory activity, was found to be responsible for the sensitizing activity of racemic etodolac. Therefore, the present invention also provides a method to treat other forms of cancer, such as breast, prostate and colon cancer with RS or the R(−) enantiomer of etodolac or an analog thereof, used alone, or preferably, in combination with a chemotherapeutic agent.

A method of evaluating the ability of etodolac to sensitize cancer cells, such as solid tumor cells or cancerous cells, such as CLL or MM cells, to a chemotherapeutic agent is also provided. The assay method comprises (a) isolating a first portion of cancer cells, such as cancer B cells or marrow plasma cells, from the blood of a human patient; (b) measuring their viability; (c) administering etodolac, R(-) etodolac or an analog thereof, to said patient; (d) isolating a second portion of cancerous cells from said patient; (e) measuring the viability of the second portion of cancerous cells; and (f) comparing the viability measured in step (e) with the viability measured in step (b); wherein reduced viability in step (e) indicates that the cells have been sensitized to said chemotherapeutic agent.

Preferably, steps (b) and (e) are carried out in the presence of the chemotherapeutic agent, as will be the case when the cells are derived from the blood or marrow of a mammal afflicted with leukemia, such as a CLL or MM patient.

Thus, a cancer patient about to undergo, or undergoing, treatment can be rapidly evaluated to see if he/she will benefit from concurrent chemotherapy and administration of etodolac or an analog thereof.

The present invention is based on the discovery by the inventors that racemic etodolac inhibits the viability of purified CLL or MM cells at concentrations that do not inhibit the viability of normal peripheral blood lymphocytes (PBLs) or normal bone marrow cells. It was then unexpectedly found that the R(−) enantiomer of etodolac is as toxic to CLL cells as is the S(+) enantiomer, although the R(−) enantiomer lacks cyclooxygenase inhibitory

activity. It was then found that etodolac synergistically interacted with fludarabine and 2-chloroadenosine to kill CLL cells at concentration at which the chemotherapeutic agents alone were inactive.

Etodolac and its analogs possess several unique disposition features due to their stereoselective pharmacokinetics. In plasma, after the administration of RS-etodolac, the concentrations of the "inactive" R-enantiomer of etodolac are about 10-fold higher than those of the active S-enantiomer, an observation that is novel among the chiral NSAIDs. See, D. R. Brocks et al., Clin. Pharmacokinet., 26, 259 (1994). After a 200 mg dose in six elderly patients, the maximum plasma concentration of the R-enantiomer was about 33 μM. In contrast, the maximum concentration of the S-enantiomer was 5-fold lower. The typical dosage of the racemic mixture of etodolac is 400 mg BID, and the drug has an elimination half-life between 6-8 hours. Thus, etodolac at commonly used dosages should achieve a plasma concentration of the R-enantiomer shown to sensitize CLL cells *in vitro* to fludarabine. Moreover, it is likely that the administration of the purified R-enantiomer will not display the side effects associated with cyclooxygenase (COX) inhibitors, such as ulcers and renal insufficiency, and thus could be given at considerably higher dosages. This is particularly advantageous in the case of MM, since cyclooxygenase inhibitors are contraindicated because they can impair renal function. It is believed that etodolac can act both directly and indirectly against cancer cells; i.e., by inhibiting factor(s) that would normally block apoptosis.

As used herein, the terms "reduce the viability of" or "sensitize" cancerous cells or "maintain the viability of normal cells" is to be understood in terms of the examples presented herein.

Brief Description of the Figures

Figure 1 is a graph depicting the sensitivity of normal peripheral blood lymphocytes (PBL) to racemic etodolac.

Figure 2 is a graph depicting the sensitivity of CLL cells to racemic etodolac.

Figure 3 is a graph depicting the synergistic effect of a combination of racemic etodolac and fludarabine against CLL cells.

Figure 4 is a graph depicting the synergistic effect of a combination of 50 μM etodolac with 10 μM 2CdA or 10 mM Fludara against CLL cells.

Figure 5 is a graph depicting the sensitivity of CLL cells to S- and R-etodolac.

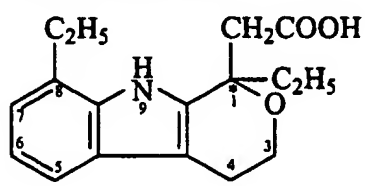
Figures 6 and 7 depict the viability of CLL cells from two patients before and after etodolac administration.

Figure 8 depicts a flow cytometric analysis of CLL cells before and after etodolac treatment.

Figures 9 and 10 depict the selective action of R(−) etodolac against MM cells from two patients.

Detailed Description of the Invention

Etodolac (1,8-diethyl-1,3,4,9-tetrahydro[3,4-6]indole-1-acetic acid) is a NSAID of the pyranocarboxylic acid class, that was developed in the early 1970s. See, C. A. Demerson et al., Ger. Pat. No. 2,226,340 (Am. Home Products); R. R. Martel et al., Can. J. Pharmacol., 54, 245 (1976). Its structure is depicted as formula (I), below, wherein (*) denotes the chiral center. See also, The Merck Index, (11th ed.), at page 608.



(I)

Early studies indicated that etodolac was an effective NSAID, with a favorable ratio of anti-inflammatory activity to adverse effects. Etodolac has been marketed for some years in Europe, including the UK, Italy, France and Switzerland, for the treatment of pain and inflammation associated with various forms of arthritis. The drug was more recently given approval for marketing in the U.S., where its approved uses are currently restricted to the treatment of osteoarthritis, and as a general purpose analgesic.

The pharmacokinetics of etodolac have been extensively reviewed by D. R. Brocks et al., Clin. Pharmacokinet., 26, 259 (1994). Etodolac is marketed as the racemate. However, Demerson et al., J. Med. Chem., 26, 1778 (1983) found that the (+)-enantiomer of etodolac possessed almost all of the anti-inflammatory activity of the racemate, as measured by reduction in paw volume of rats with

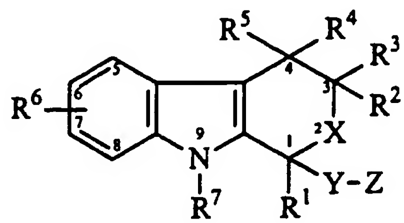
adjuvant polyarthritis, and prostaglandin synthetase inhibitory activity of the drug. No activity was discernible with the (-)-enantiomer. The absolute configurations of the enantiomers were found to be S-(+) and R-(-), which is similar to that for most other NSAIDs. The enantiomer of the racemate can be resolved by fractional crystallization using optically active 1-phenylethylamine, and use of HPLC to determine racemic etodolac and enantiomeric ratios of etodolac and two hydroxylated metabolites has been reported by U. Becker-Scharfenkamp et al., J. Chromatog., 621, 199 (1993) and B. M. Adger et al. (U.S. Patent No. 5,811,558).

The *in vivo* disposition of etodolac is extremely stereoselective, with plasma concentrations of the "inactive" R-enantiomer greatly exceeding those of the "active" S-enantiomer. In this respect, etodolac is unique in relation to other chiral NSAIDs, for which the "active" S-enantiomer usually attains plasma concentrations that are similar to or higher than those of the "inactive" enantiomer. Nonetheless, the R(-) enantiomer has been asserted to have some analgesic activity. See, Young et al., U.S. Patent No. 5,561,151. However, as exemplified below, this unusual disposition facilitates administration of amounts of etodolac that are effective to sensitize leukemic cells to chemotherapeutic agents, without giving rise to the side effects of the "active" anti-inflammatory S(+) enantiomer.

The chemical synthesis of the racemic mixture of etodolac can be performed by the method described in Demerson et al., U.S. Patent No. 3,843,681; and C.A. Demerson et al., J. Med. Chem., 19(3), 391 (1976), the disclosures of which are hereby incorporated by reference.

The R(-) isomer of etodolac may be obtained by resolution of the mixture of enantiomers of etodolac using conventional means, such as the formation of a diastereomeric salt with a optically active resolving amine; see, for example, "Stereochemistry of Carbon Compounds," by E. L. Eliel (McGraw Hill, 1962); C. H. Lochmuller et al., J Chromatog., 113, 283 (1975); "Enantiomers, Racemates and Resolutions," by J. Jacques, A. Collet, and S. H. Wilen, (Wiley-Interscience, New York, 1981); and S. H. Wilen, A. Collet, and J. Jacques, Tetrahedron, 33, 2725 (1977).

Analogs of etodolac that can be used in the present inventions are disclosed *inter alia*, in C. A. Demerson (U.S. Patent No. 3,843,681), as compounds of formula (II):



in which R^1 is selected from the group consisting of lower alkyl, lower alkenyl, lower alkynyl, lower cycloalkyl, phenyl, benzyl and 2-thienyl, R^2, R^3, R^4 and R^5 are the same or different and are each selected from the group consisting of hydrogen and lower alkyl, R^6 is selected from the group consisting of hydrogen, lower alkyl, hydroxy, lower alkoxy, benzyloxy, lower alkanoyloxy, nitro and halo, R^7 is selected from the group consisting of hydrogen, lower alkyl and lower alkenyl, X is selected from the group consisting of oxy and thio, Y is selected from the group consisting of carbonyl or $(C_1\text{-}C_3)$alkylC(O), wherein each alkyl is substituted with 0-2 $(C_1\text{-}C_4)$alkyl and Z is selected from the group consisting of hydroxy, lower alkoxy, amino, lower alkylamino, di(lower)alkylamino and phenylamino, or a pharmaceutically acceptable salt thereof. Lower (alkyl, alkenyl, alkanoyl, etc.) indicates a $C_1\text{-}C_6$ group, preferably a $C_1\text{-}C_4$ group.

The magnitude of a prophylactic or therapeutic dose of racemic or R-etodolac in the acute or chronic management of cancer, i.e., a leukemia such as CLL or MM, will vary with the stage of the cancer, such as the solid tumor or leukemia to be treated, the chemotherapeutic agent(s) or other anti-cancer therapy used, and the route of administration. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. In general, the total daily dose range for racemic or R-etodolac, for the conditions described herein, is from about 50 mg to about 5000 mg, in single or divided doses. Preferably, a daily dose range should be about 100 mg to about 4000 mg, most preferably about 1000-3000 mg, in single or divided doses, e.g., 750 mg every 6 hr of orally administered R(−)-etodolac. This can achieve plasma levels of about 500-750 μM, which can be effective to

kill leukemic cells. In managing the patient, the therapy should be initiated at a lower dose and increased depending on the patient's global response. It is further recommended that infants, children, patients over 65 years, and those with impaired renal or hepatic function initially receive lower doses, and that they be titrated based on global response and blood level. It may be necessary to use dosages outside these ranges in some cases. Further, it is noted that the clinician or treating physician will know how and when to interrupt, adjust or terminate therapy in conjunction with individual patient response. The terms "an effective amount" or "an effective sensitizing amount" are encompassed by the above-described dosage amounts and dose frequency schedule.

Any suitable route of administration may be employed for providing the patient with an effective dosage of etodolac, i.e., R(-)etodolac. For example, oral, rectal, parenteral (subcutaneous, intravenous, intramuscular), intrathecal, transdermal, and like forms of administration may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, patches, and the like. The etodolac may be administered prior to, concurrently with, or after administration of chemotherapy, or continuously, i.e., in daily doses, during all or part of, a chemotherapy regimen, such as a melphalan regimen, optionally with total body irradiation and subsequent bone marrow transplant or stem cell transplant. The etodolac, in some cases, may be combined with the same carrier or vehicle used to deliver the anti-cancer chemotherapeutic agent.

Thus, the present compounds may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit

dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrated agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. a syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a non-toxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water,

ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, non-toxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Patent No. 4,938,949.

The invention will be further described by reference to the following detailed examples.

Example 1. Sensitivity of Normal Peripheral Blood Lymphocytes and CLL Cells to Etodolac

Mononuclear cells were isolated from the peripheral blood of B-CLL patients and normal donors using density gradient centrifugation (Ficoll-Paque). Cells were cultured at 2×10^6 cells per mL in RPMI with 20% autologous plasma in 96-well plates with or without the indicated μM concentrations of etodolac (racemic, S-etodolac, R-etodolac) and in combination with 2-chloro-2'-deoxyadenosine (2CdA) or fludarabine. At indicated times (12, 24, 36, 48, 60,

72 hours), viability assays were performed using the erythrocin B exclusion assay, as described by D. Carson et al., PNAS USA, 89, 2970 (1992).

As shown in Figure 1, significant death of normal PBLs occurred only at 800 μM racemic etodolac, a concentration which cannot be obtained *in vivo*.

Peripheral blood lymphocytes from a normal donor were cultured with 1.0 mM etodolac for 24 hours. Then B lymphocytes were identified by staining with anti-CD19 antibody, and viability was assessed by $DiOC_6$ fluorescence. Etodolac under these conditions did not reduce the viability of the normal B cells, compared to control cultures. When the same viability assay was run with purified CLL cells from the peripheral blood of a CLL patient, the results were different. As shown in Figure 2, 50% of the CLL cells were killed by a 48 hour exposure to 200 μM racemic etodolac. More than 95% of the treated cells were malignant B lymphocytes.

Example 2. Synergistic Combinations of Etodolac and Chemotherapeutic Agents

Fludarabine is a nucleoside analog commonly used for the treatment of CLL. In this experiment the *in vitro* survival of CLL cells at the indicated time points was compared in cultures containing medium alone ("Con", squares), fludarabine 10 nM (diamonds), etodolac 10 μM (closed circles), and fludarabine 10 nM plus etodolac 10 μM (open circles). The two drugs together exhibited a synergistic cytotoxic effect. Figure 3 shows that the combination killed 50% of CLL cells during 48 hours of culture, while either drug alone was ineffective. Figure 4 demonstrates synergy between 50 μM etodolac and 10 nM 2-chlorodeoxyadenosine and fludarabine, under the same test conditions.

Example 3. Effect of R(-) and S(+) Etodolac Against CLL Cells

Etodolac tablets were ground in a mortar and extracted from the formulation using ethyl acetate. The resulting racemic mixture of enantiomers was separated into R and S isomers on a preparative scale by fractional crystallization by the procedure of Becker-Scharfenkamp and Blaschke, J. Chromatog., 621, 199 (1993). Thus, the racemic mixture solid was dissolved in absolute 2-propanol and S-1-phenylethylamine was added to the solution. The resulting salt solution was stored in the refrigerator for 4 days. The crystalline white salt product was filtered and washed with cold 2-propanol and

recrystallized two more times from 2-propanol. The same procedure was repeated for the R isomer only using R-1-phenylethylamine as the resolving agent. Finally, the R and S salts were decomposed using 10% sulfuric acid (v/v) and extracted with ethyl acetate. The chiral purity of each isomer was verified by HPLC using a Chiral-AGP column from ChromTech.

The toxicities of the two enantiomers to CLL cells cultured in RPMI 1640 medium with 10% autologous plasma were compared at the indicated concentrations and time points, as shown in Figure 5. The R- and S-enantiomers are equivalently cytotoxic to the CLL cells.

Example 4. Viability of CLL Cells Before and After Etodolac Treatment

Heparinized blood was taken from two patients (JK and NA) with CLL. Then each patient immediately took a 400 mg etodolac tablet, and a second tablet 12 hours later. After another 12 hours, a second blood specimen was obtained. The CLL cells were isolated and their survival *in vitro* were compared in RPMI 1640 medium containing 10% autologous plasma, as described in Example 1. The circles show CLL cells before etodolac treatment. In Figures 6-7, the upward pointing triangles represent CLL cell viability after etodolac treatment, wherein the cells are dispersed in medium containing the pretreatment plasma. The downward pointing triangles are CLL cells after treatment maintained in medium with the post-treatment plasma.

Figure 6 shows the different survivals of the two cell populations from patient JK. Note that the cells after treatment had a shortened survival compared to the cells before treatment. Figure 7 shows a less dramatic but similar effect with patient NA. Figure 8 is a flow cytometric analysis of CLL cells from patient JK before and after etodolac treatment. $DiOC_6$ is a dye that is captured by mitochondria. When cells die by apoptosis, the intensity of staining decreases. The X axis on the four panels in Figure 8 shows the $DiOC_6$ staining. An increased number of dots in the left lower box indicates cell death by apoptosis. If one compares the cells taken from the patient before etodolac treatment, and after etodolac treatment, one can see that the number of dots in the left lower box is much higher after the drug. This effect is detectable at 12 hours, and increases further after 24 hours.

To conduct the flow cytometric analysis, the mitochondrial transmembrane potential was analyzed by 3,3' dihexyloxacarboncyanide iodide ($DiOC_6$), cell membrane permeability by propidium iodide (PI)[3] and mitochondrial respiration by dihydrorhodamine 123 (DHR) (See J. A. Royall et al., Arch. Biochem. Biophys., 302, 348 (1993)). After CLL cells were cultured for 12 or 24 hours with the indicated amount of etodolac, the cells were incubated for 10 minutes at 37° C in culture medium containing 40 nM of $DiOC_6$ and 5 μg/ml PI. Cells were also cultured for 3 hours with the indicated amount of etodolac, spun down at 200 × g for 10 minutes and resuspended in fresh respiration buffer (250 mM sucrose, 1 g/L bovine serum albumin, 10 mM $MgCl_2$, 10 mM K/Hepes, 5 mM KH_2PO_4 (pH 7.4)) and cultured for 10 minutes at 37° C with 0.04% digitonin. Then cells were loaded for 5 minutes with 0.1 μM dihydrorhodamine (DHR). Cells were analyzed within 30 minutes in a Becton Dickinson FAC-Scalibur cytofluorometer. After suitable comprehension, fluorescence was recorded at different wavelength: $DiOC_6$ and DHR at 525 nm (Fl-1) and PI at 600 nm (FL-3).

As a general matter a reduction of 10% in the survival of the post-treatment malignant cells, compared to the pretreatment malignant cells, at 16 hours after culture *in vitro* is considered a "positive" in this test, and indicates the use of etodolac, i.e., R(−) etodolac in CLL or other cancer therapy.

Example 5. Ability of R(−)-Etodolac to Selectively Kill MM Cells

Bone marrow was obtained from two patients with multiple myeloma. The marrow contained a mixture of malignant cells, as enumerated by high level expression of the CD38 membrane antigen, and normal cells. The suspended marrow cells were incubated for 72 hours in RPMI 1640 medium with 10% fetal bovine serum, and various concentrations of the purified R-enantiomer of etodolac. Then the dead cells were stained with propidium iodide, and the multiple myeloma cells were stained with fluorescent monoclonal anti-CD38 antibodies. The data were analyzed by fluorescent activated cell sorting. Figures 9-10 show that R-etodolac did not kill the normal bone marrow cells (light bars), but dose-dependently killed the multiple myeloma cells (dark shaded areas), in the marrow cells from both patients.

All of the publications and patent documents cited hereinabove are incorporated by reference herein. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

What is claimed is:

1. A method to treat multiple myeloma comprising contacting mammalian cancer cells with an amount of etodolac or an analog thereof, effective to reduce the viability of cancerous bone marrow cells.

2. A method of increasing the susceptibility of mammalian cancer cells to killing by a chemotherapeutic agent comprising contacting the cells with an effective sensitizing amount of etodolac or an analog thereof.

3. The method of claim 1 or 2 wherein the mammalian cancer cells are human cancer cells.

4. The method of claim 3 wherein the etodolac or the analog thereof is administered to a human cancer patient.

5. The method of claim 2 wherein the cancer is a leukemia.

6. The method of claim 5 wherein the leukemia is chronic lymphocytic leukemia.

7. The method of claim 5 wherein the leukemia is multiple myeloma.

8. The method of claim 4 wherein the cancer patient is undergoing treatment with a chemotherapeutic agent.

9. The method of claim 2 wherein the cancer cells are cells of a solid tumor.

10. The method of claim 9 wherein the cancer cells are prostate cancer cells.

11. The method of claim 4, wherein the etodolac is R(−) etodolac.

12. The method of claim 6, 7, 9 or 10 wherein the etodolac is R(−) etodolac.

13. The method of claim 4 wherein the etodolac or analog thereof is administered orally.

14. The method of claim 11 wherein the R(−) etodolac is administered orally.

15. The method of claim 12 wherein the R(−) etodolac is administered orally.

16. The method of claim 5 wherein the etodolac or analog thereof is administered in combination with melphalan.

17. The method of claim 16 wherein the R(−) etodolac is given in combination with melphalan.

18. The method of claim 16 wherein a dose of about 140-200 mg/m^2 melphalan is given in combination with R(−) etodolac.

19. A method of evaluating the ability of etodolac to sensitize cancer cells to a chemotherapeutic agent comprising:

(a) isolating a first portion of cancer cells from a human leukemia patient;

(b) measuring their viability;

(c) administering etodolac to said patient;

(d) isolating a second portion of cancer cells from said patient;

(e) measuring the viability of the second portion of cancer cells; and

(f) comparing the viability measured in step (e) with the viability measured in step (b); wherein reduced viability in step (e) indicates that the cells have been sensitized to said chemotherapeutic agent.

20. The method of claim 19 wherein steps (b) and (e) are carried out in the presence of the chemotherapeutic agent.

21. The method of claim 19 wherein the cancer cells are chronic lymphocytic leukemia cells.

22. The method of claim 19 wherein the cancer cells are multiple myeloma cells.

23. The method of claim 19 wherein the etodolac is R(−) etodolac.

24. The method of claim 19 or 20 wherein the chemotherapeutic agent is Fludara or 2CdA.

25. The method of claim 19 or 20 wherein the chemotherapeutic agent is melphalan.

26. A method of treating multiple myeloma comprising administering to a human patient afflicted therewith an amount of R(−) etodolac effective to reduce the viability of multiple myeloma cells, while maintaining the viability of normal bone marrow cells.

27. The method of claim 26 wherein the R(−) etodolac is administered orally.

28. The method of claim 26 wherein a daily dosage of about 1000-5000 mg of R(−) etodolac is administered.

29. The method of claim 28 wherein the plasma concentration of etodolac is about 500-700 μM.

30. The method of claim 26 further comprising treating the patient with melphalan.

31. The method of claim 26 wherein at least one dosage of about 140-200 mg/m^2 of melphalan is administered.

32. The method of claim 30 further comprising treating the patient with total body irradiation.

33. The method of claim 30 or 32 further comprising administering a bone marrow or stem cell transplant to the patient.

Figure 1

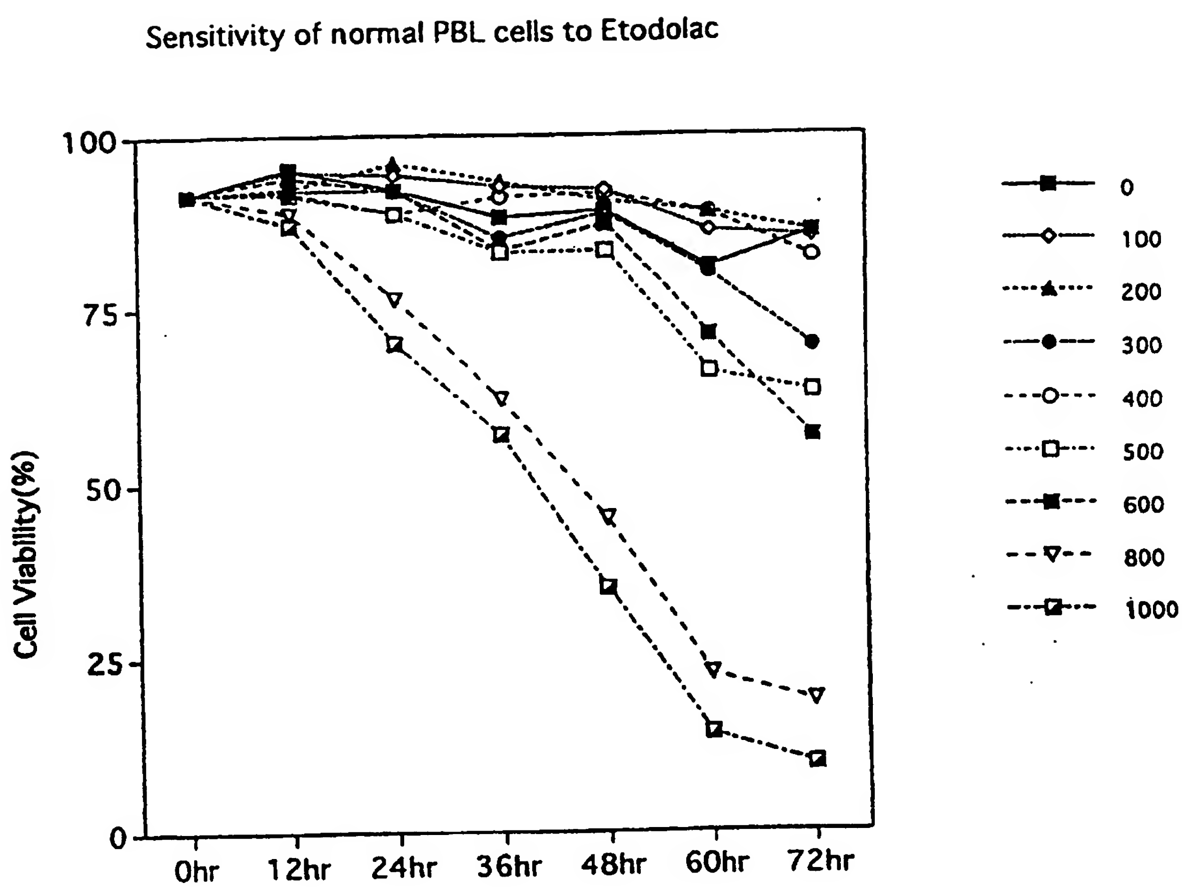

Sensitivity of normal PBL cells to Etodolac
Cell Viability(%)
100
75
50
25
0
0hr
12hr
24hr
36hr
48hr
60hr
72hr
0
100
200
300
400
500
600
800
1000

Figure 2

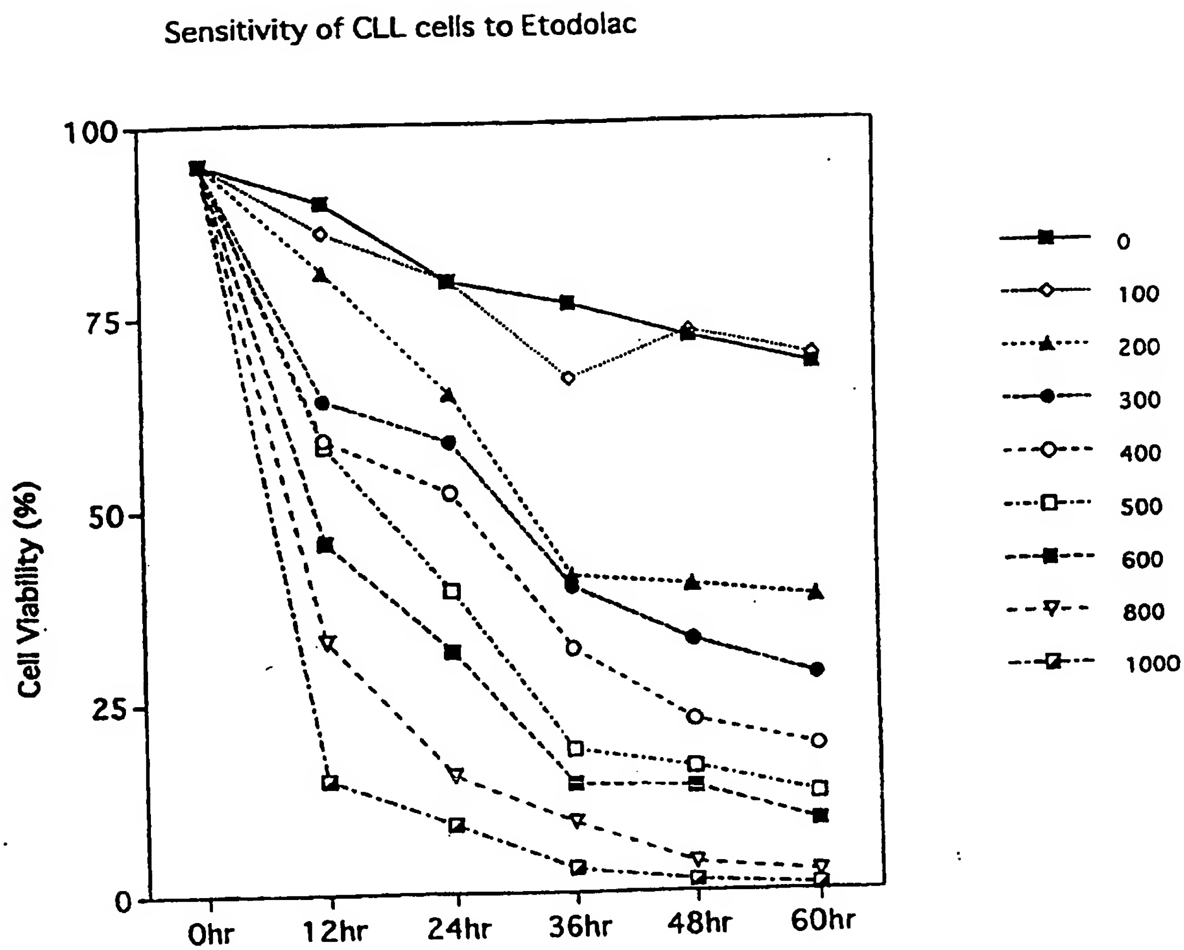

Sensitivity of CLL cells to Etodolac
Cell Viability (%)
100
75
50
25
0
0hr
12hr
24hr
36hr
48hr
60hr
0
100
200
300
400
500
600
800
1000

Figure 3

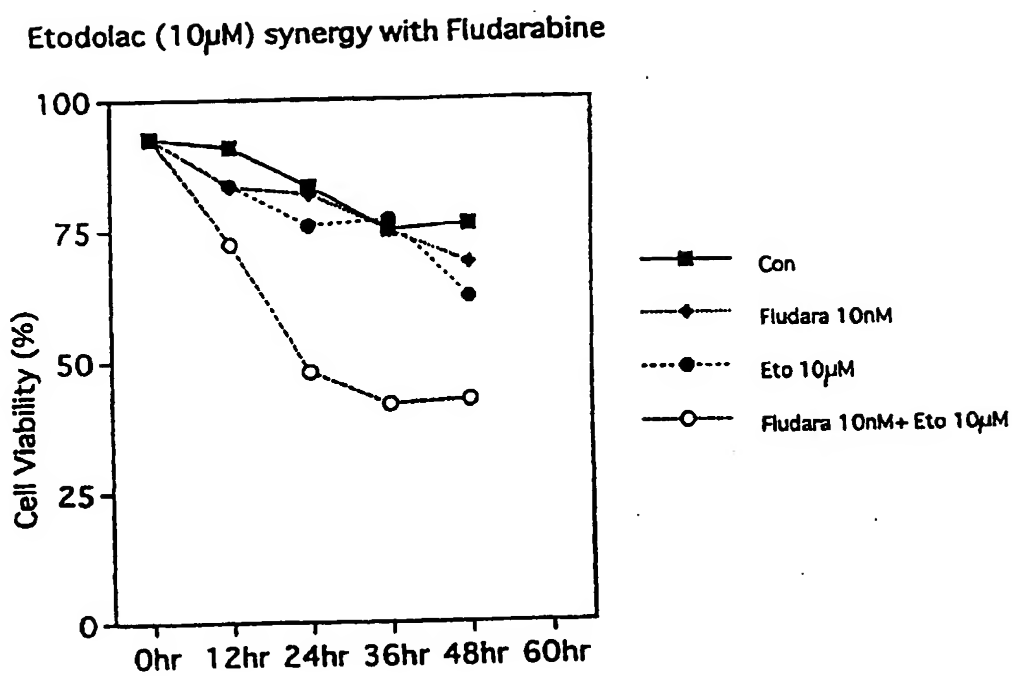

Etodolac (10μM) synergy with Fludarabine
Cell Viability (%)
100
75
50
25
0
0hr
12hr
24hr
36hr
48hr
60hr
Con
Fludara 10nM
Eto 10μM
Fludara 10nM+ Eto 10μM

Figure 4

Etodolac (50μM) synergy with 2CdA and Fludarabine

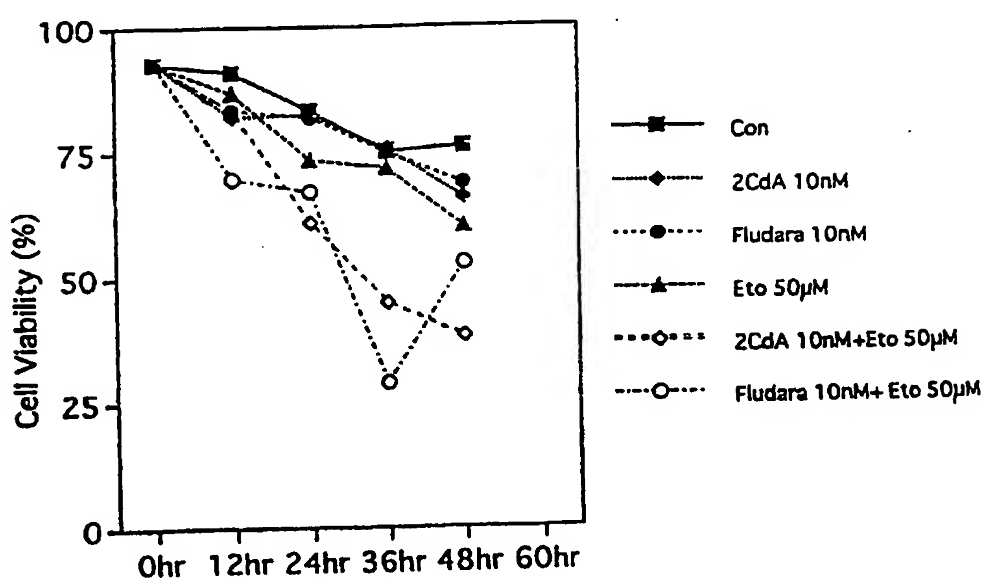


Figure 5

Sensitivity of CLL cells to S-Etodolac and R-Etodolac

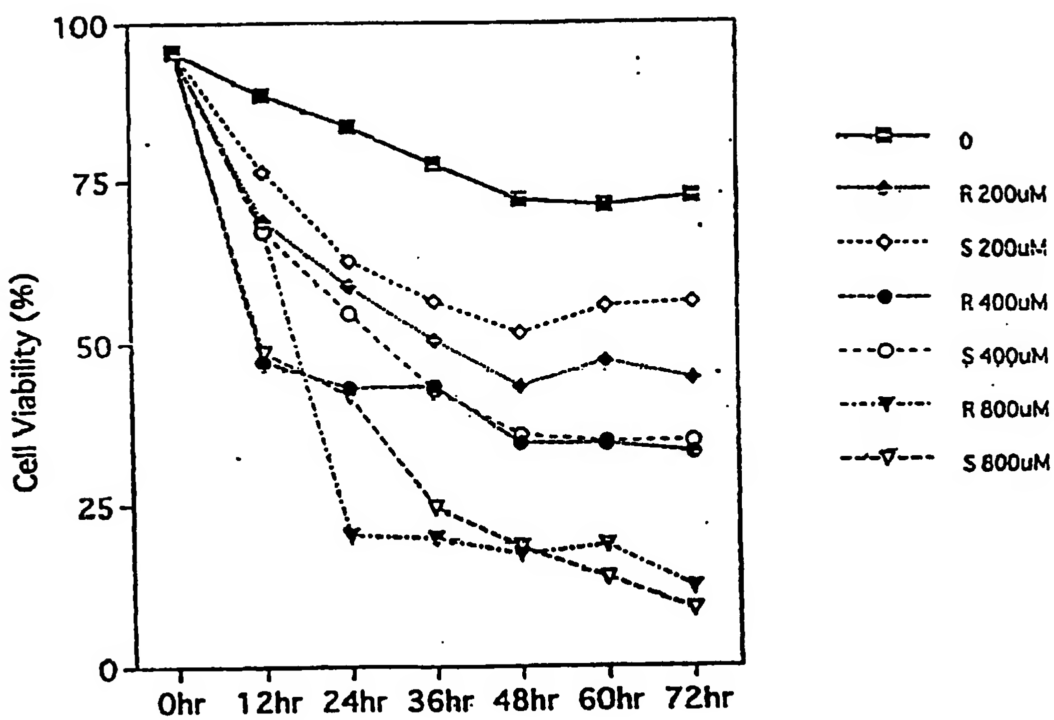


Figure 6

Cell Viability of CLL cells (J.K.) before and after Etodolac treatment

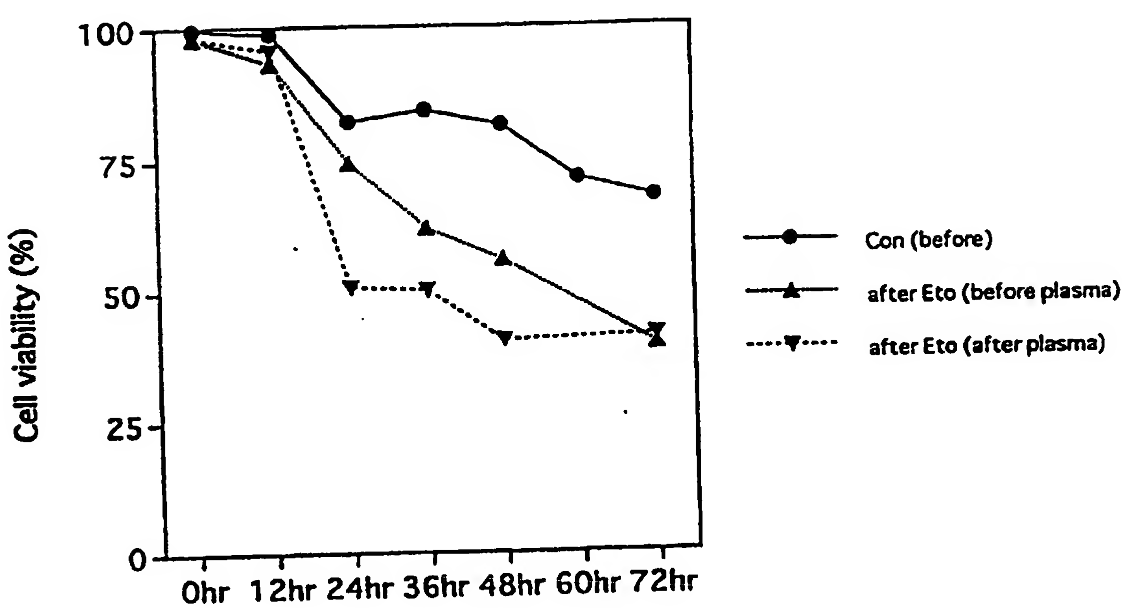


Figure 7

Cell Viability of CLL cells (N.A.) before and after Etodolac treatment

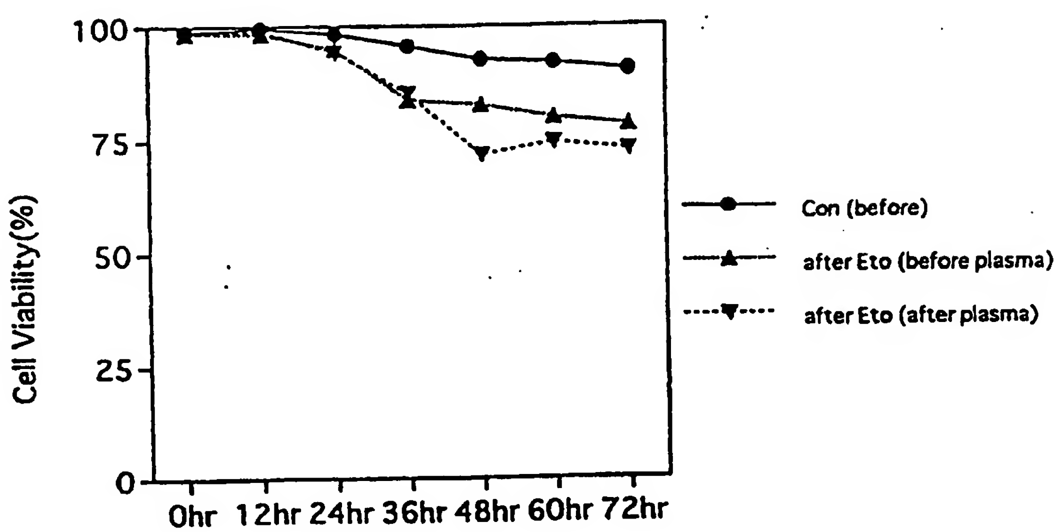
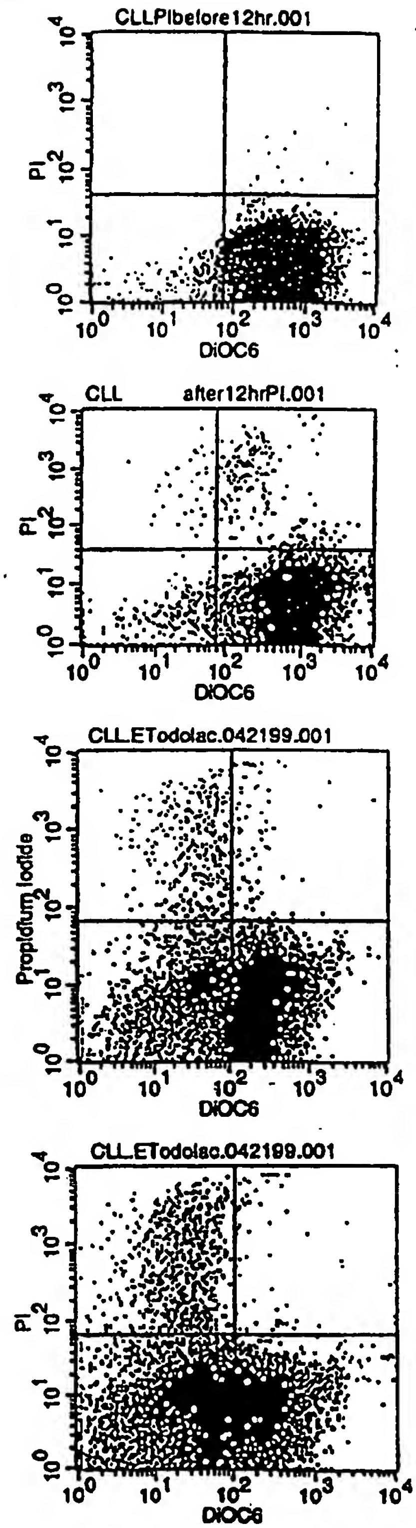


Figure 8



12hr before

12hr after

24hr before

24hr after

Fig. 9

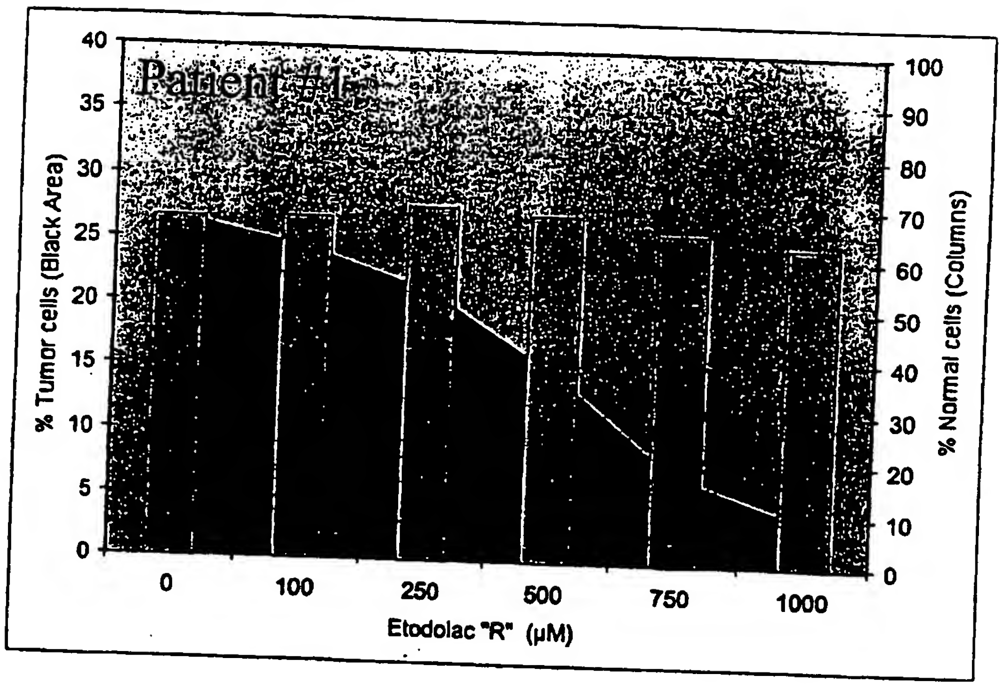
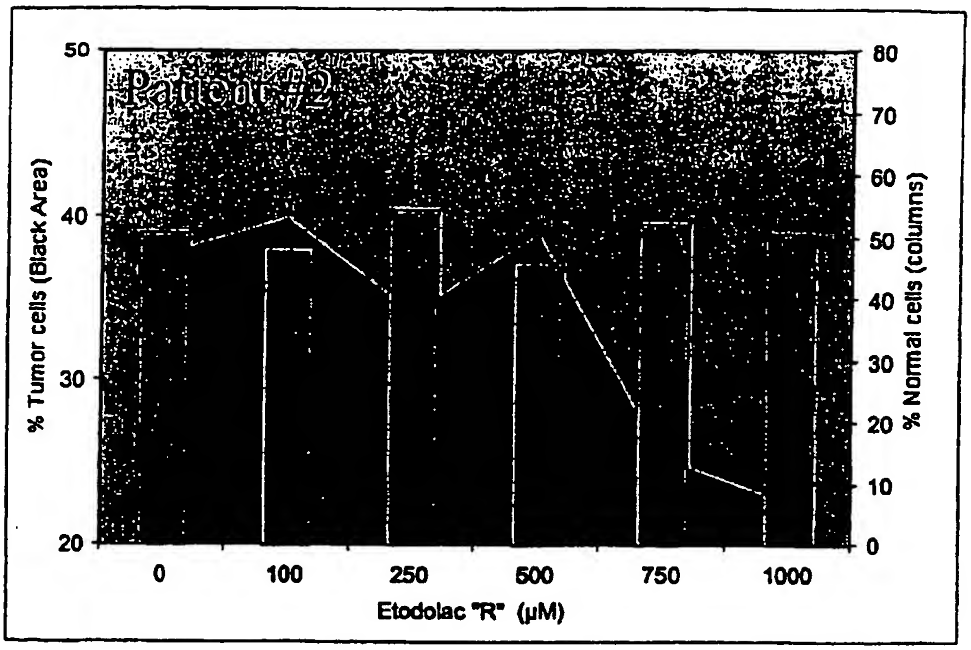

Patient #1
% Tumor cells (Black Area)
% Normal cells (Columns)
0 100 250 500 750 1000
Etodolac "R" (μM)
40 35 30 25 20 15 10 5 0
100 90 80 70 60 50 40 30 20 10 0

Fig. 10


Patient #2
% Tumor cells (Black Area)
% Normal cells (columns)
50
40
30
20
80
70
60
50
40
30
20
10
0
0
100
250
500
750
1000
Etodolac "R" (µM)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 February 2001 (01.02.2001)

PCT

(10) International Publication Number
WO 01/06990 A3

(51) International Patent Classification⁷: A61K 31/40

(21) International Application Number: PCT/US00/40370

(22) International Filing Date: 13 July 2000 (13.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/360,020 23 July 1999 (23.07.1999) US
09/589,476 7 June 2000 (07.06.2000) US

(71) Applicant *(for all designated States except US)*: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 12th Floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: CARSON, Dennis, A. [US/US]; 14824 Vista del Oceano, Del Mar, CA 92014 (US). COTTAM, Howard, B. [US/US]; 1890 Lendee Drive, Escondido, CA 92025 (US). ADACHI, Souchi [JP/US]; 8223 Caminito Modena, La Jolla, CA 92037 (US). LEONI, Lorenzo, M. [CH/US]; 3977 Camino Calma, San Diego, CA 92122 (US).

(74) Agent: VIKSNINS, Ann, S.; Schwegman, Lundberg, Woessner & Kluth, P.O. Box 2938, Minneapolis, MN 55402 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report: 26 April 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/06990 A3

(54) Title: USE OF ETODOLAC TO TREAT CANCER

(57) Abstract: A method of treating multiple myeloma (MM) is provided comprising administering an amount of etodolac to a subject afflicted with MM that is effective to selectively reduce the viability of and/or sensitize the cancer cells to an anti-cancer agent.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/40370

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/40
US CL :514/411
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/411

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T,P	WO 00/02555 A1 (NARDELLA ET AL.)20 January 2000.	1-33
X	WO 96/28148 A2 (LOMA LINDA UNIVERSITY MEDICAL CENTER) 19 September 1996), see entire document.	1-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
01 DECEMBER 2000

Date of mailing of the international search report
22 JAN 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
DMC
DELLA MAE COLLINS
PARALEGAL SPECIALIST
TECHNOLOGY CENTER 1600
JEROME D. GOLDBERG
Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☑ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: ______________________

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.